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14. ABSTRACT

BMP activity is tightly regulated at extracellular and intracellular levels of signaling as the dosage of BMPs can have widely varied effects depending on the time, tissue and domain in which they are expressed. However, the regulation of BMP signaling within the MG, both during normal development and oncogenesis, remains largely unknown. Twisted gastrulation (TWSG1) is a conserved extracellular modulator of BMPs. It can promote or inhibit BMP signaling depending on the constituents of the extracellular milieu. Twsg1 is expressed in mammary glands from wild type (WT) mice at 3, 6 and 8 weeks of life, and is differentially expressed in breast cancer, yet its role in MG development and breast cancer pathogenesis is unknown. This study is designed to determine how TWSG1 regulates the invasion of normal MG tissue during ductal elongation and the behavior of breast cancer cells.

15. SUBJECT TERMS
BMP, Mammary gland development, ductal elongation, breast cancer

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Introduction

It has been shown that BMP expression is differentially regulated in breast cancer and understanding the role BMPs play in cancer is an active area of research (1, 2). Since many developmental programs are co-opted or misregulated in tumorigenic cells, it is critical that we understand the role these molecules play during development (3, 4). Twisted gastrulation, an extracellular BMP binding protein, can modulate BMP signaling in a variety of tissues (5-7). We therefore decided to use a *Twsg1*$^{-/-}$ mouse model to first investigate the role of BMP binding proteins in mammary gland (MG) development and secondly to understand their role in breast cancer. Our preliminary data identified abnormal postnatal mammary gland morphogenesis in *Twsg1*$^{-/-}$ mice. Our current data demonstrates a role for TWSG1 in the regulation apoptosis in breast cancer (BC).

Body

The experiments outlined in Aim 1 and Aim 2 were completed and reported in the Annual Report submitted March 2012. Furthermore, data from those experiments were published in Forsman et al, Dev. Biol. 2013 listed in the Reportable Outcomes section of this report.

Our preliminary data demonstrated overexpression of TWSG1 in BC by microarray (data not shown.) However, there was no clear correlation between the molecular subtype and TWSG1 expression in the patient population sampled. Immunohistochemistry demonstrated that TWSG1 was expressed in what appeared to be the myoepithelium of the normal breast and was expressed in a wide variety of BCs (Figure 1).

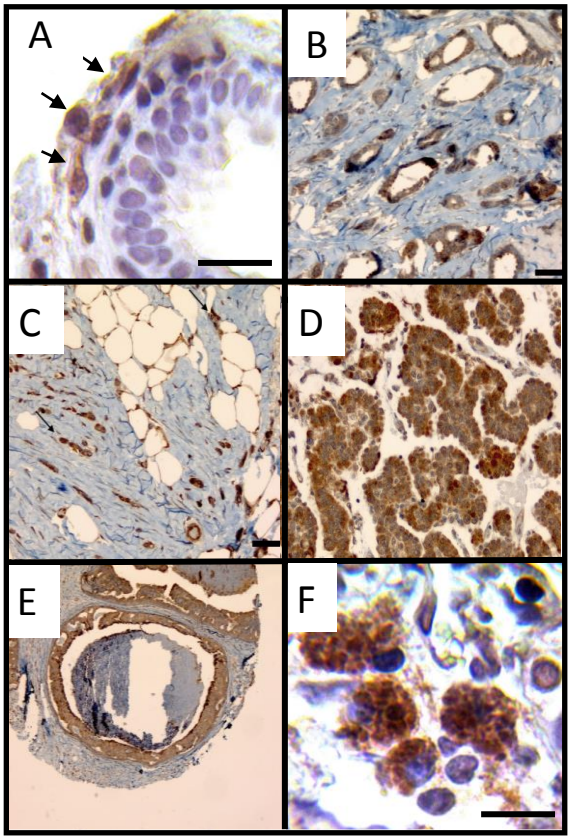

Figure 1. TWSG1 is expressed in a variety of breast cancers. A.) TWSG1 seems to be restricted to the myoepithelium. Expression is detected in B.) invasive ductal carcinoma C.) invasive lobular carcinoma D.) invasive papillary carcinoma E.) ductal carcinoma in situ F.) papillary carcinoma

Specific Aim 3: To examine if TWSG1 is involved in breast cancer pathogenesis. **Task 1: Examine the expression of *Twsg1, Bmp2, Bmp4, Bmp7, Bmprla* and *Bmprlb, Bmprll* in ER positive/PR positive and triple negative breast cancer cell lines.** We were able to determine that in all breast cancer cells (BCC) lines examined (MCF10A, SKBR3, HCC1569, MD-MBA-231, T47D and MCF7) transcripts for a complete BMP pathway were present and that *TWSG1* was expressed in all. When transcripts were quantified by QPCR, MD-MBA-231 and T47D expressed significantly higher levels of

TWSG1 while all others tested expressed the same levels as MCF10A (data not shown.) We chose to move forward with the MD-MBA-231 and select other triple negative breast cancer cells (TNBCC) in which to study the role of TWSG1. Interestingly, we observed by RTPCR a pattern of ligand transcript switching from BMP2 to BMP4 and a switch from the expression of Chordin (CHRD) to that of Chordinlike 1 (CHRDL1) both are extracellular BMP binding proteins that can bind BMP and TWSG1 (data not shown). To further confirm ligand transcript change we completed QPCR in MCF10As and MD-MBA-231s. MCF10As express BMP2 transcripts while MD-MBA-231s do but at a very low level and instead express BMP4 transcripts at a high level while MCF10As do not. (Figure 2.)

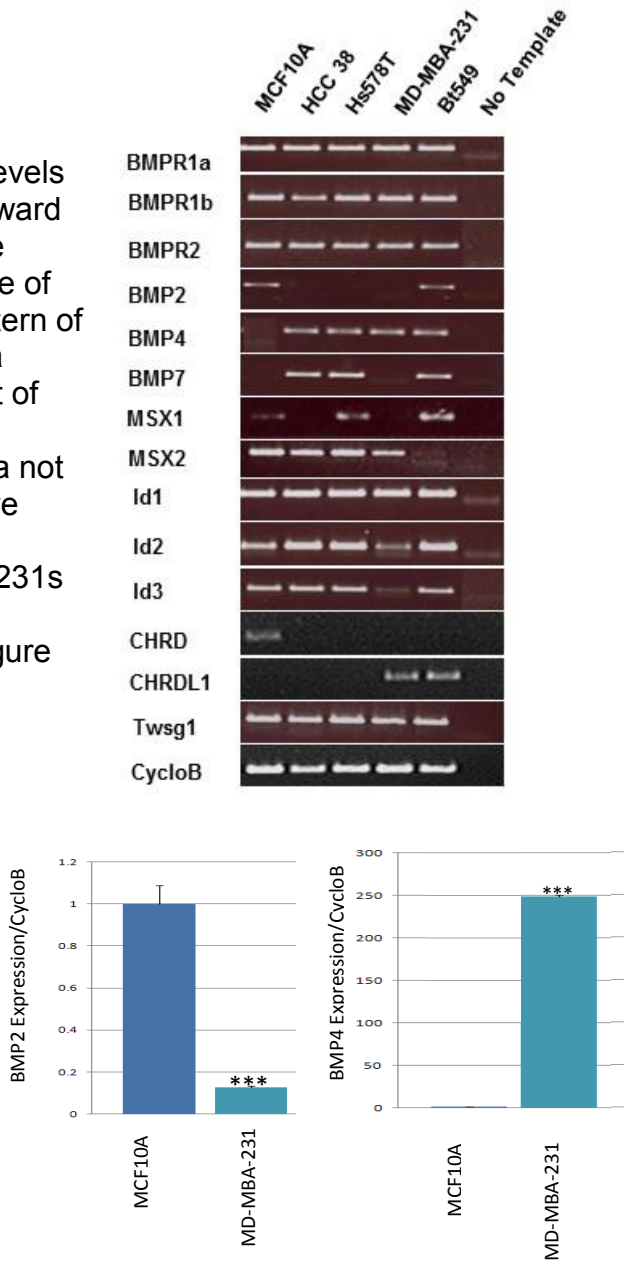

Figure 2. BMP pathway components are expressed in a variety of breast cancer cell lines. A.) RTPCR of BMP pathway components in four TNBCCs lines and normal breast cell line B.) QPCR of BMP2 and BMP4 in normal and the TNBCC line MD-MBA-231.

Task 2: Examine if overexpression or silencing of Twsg1 in breast cancer cell lines affects BMP signaling level.

Cells were transduced with adenovirus carrying either a CMV-EGFP cassette or a double cassette of CMV-EGFP, CMV-hTWSG1.Transduced cells were plated onto chamber slides and stained with anti-pSMAD1/5/8. There were significantly more cells in the TWSG1 transduced cells with pSMAD localized to the nucleus (data not shown.) It was also observed that that there was an increase in cell size and an increase in the cytoplasmic to nuclear ratio. Quantification of pSMAD by western blot is currently in progress.

Task 3: Determine if altered BMP level affects migration of breast cancer cells.

A transwell migration assay was used to determine if altering the levels of TWSG1 expression could change the migratory behavior of normal breast and BCCs. Cells were serum starved and then placed into migration chambers with 2% serum as a chemoattractant. The normal cell line, MCF10A, overexpressing TWSG1 was able to migrate significantly better than control tranduced (Figure 3.)

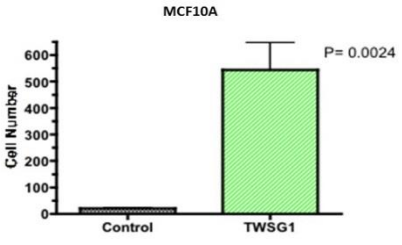

Figure 3. TWSG1 increase migratory behavior. A.) TWSG1 transduced cells migrate significantly more than control transduced cells.

Given BMPs role in regulating apoptosis we investigated

apoptosis in control and TWSG1 transduced cells by Apoptox assay and FLOW using Annexin V staining. TWSG1 overexpression in normal, MCF10A, cells led to an increase in apoptosis while overexpression in the TNBCC line MD-MBA-231 led to a reduction in apoptosis (data not shown.) We have begun studies to assess proliferation and invasion.

Key Research Accomplishments

- **TWSG1 promotes BMP signaling in normal and breast cancer cells.**
- **TWSG1 promotes migration in normal breast cells.**
- **TWSG1 promotes apoptosis in normal breast cells.**

Mentorship and Development

I have attended Dr. Kaylee Schwertfeger's weekly lab meetings and have met with Dr. Douglas Yee once a semester to discuss findings. I have attended breast cancer journal club and have presented at these meetings. I attended the Mammary Gland Gordon Conference and the Society of Developmental Biologists' Annual Meeting where I presented a poster of my work and received critical feedback. The Gordon Conference allowed me to discuss my findings one on one with leaders in the field which dramatically enhanced my understanding and refined my work significantly.

Reportable Outcomes

Publications:

Forsman CL, Ng BC, Heinze RK, Kuo C, Sergi C, Gopalakrishnan R, Yee D, Graf D, Schwertfeger KL, Petryk A. BMP-binding protein twisted gastrulation is required in mammary gland epithelium for normal ductal elongation and myoepithelial compartmentalization. Dev Biol. 2013 Jan 1;373(1):95-106

Abstracts

Forsman C, Ng B, Heinze R, Kuo C, Sergi C, Gopalakrishnan R, Yee D, Graf D, Schwertfeger K, Petryk A. Twisted Gastrulation regulates BMP signaling in the postnatal MG. Gordon Research Conference on Mammary Gland Biology, Barga, Italy, June 2012.

Forsman C, Ng B, Heinze R, Kuo C, Sergi C, Gopalakrishnan R, Yee D, Graf D, Schwertfeger K, Petryk A. Twisted Gastrulation regulates BMP signaling in the postnatal MG. Society for Developmental Biology Conference. Montreal, Canada. July 2012

Conclusion

This award has supported work that has identified a role for BMP signaling in the postnatal MG. Furthermore, our work has identified TWSG1 as an agonist of BMP signaling for the first time in a mammalian system. BMP signaling is often misregulated in cancer and understanding the role it plays during development will help inform how it might play a role in tumorigenesis. To investigate TWSG1 role in BC further we stained for TWSG1 in breast cancer tissue and have shown that TWSG1 is expressed in this tissue as well as the normal breast. We were able to show that a complete BMP pathway is transcribed in these cells. Curiously, we identified a change in ligand transcripts between normal and BCC as well as a difference in extracellular partners. We currently have studies in progress that have shown an increase in apoptosis in normal overexpressing cells and a reduction in apoptosis in MD-MBA-231 overexpressing cells. Altogether, these data are beginning to highlight the importance of BMP signaling in regulating apoptosis in BC.

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